RAS Lifesciences Pvt Ltd
Striving for better health

RAS RT Enzy

(MMLV Reverse Transcriptase)

Description

Moloney Murine Leukemia Virus Reverse Transcriptase (MMLV RT) is a RNA dependent DNA polymerase that uses single-stranded RNA as template in the presence of primer/Oligo dT to synthesize a complementary DNA strand. It can synthesize cDNA ranging from 100 bp to 3 kb.

Source

Produced from an *E. coli* strain that possesses the Reverse transcriptase gene from MMLV.

Applications

- 1st strand cDNA synthesis
- RT-PCR
- Primer extension

Supplied in: 20 mM Tris-HCl (pH 7.5), 100 mM NaCl, 0.1 mM EDTA, 1 mM DTT, 0.2% (v/v) Triton-x 100, 50% (v/v) glycerol

Concentration

100 U/µL

Unit Definition

One unit incorporates 1 nmol of dTTP into acid-precipitable material in 10 min at 37°C using poly(A)•oligo(dT)as template-primer.

Reagents Supplied with Enzyme

0.1 M DTT
5X RAS RT buffer

- 250mM Tris-HCl (pH 8.3)
- 250mM KCl
- 20mM $MgCl_2$

Storage conditions

- The recommended storage condition is -20°C for supplied components (non-frost free).
- Thaw the components prior to use and immediately refreeze after use.

Assay Conditions

First-Strand cDNA Synthesis

Add the following components to a nuclease-free microcentrifuge tube

Components	Volume (µL)
Extracted RNA (1 ng-5 µg of RNA or 1-500 ng of mRNA)	variable
50-250 ng Random primers/10 pmol Specific primer/200-500 ng oligo (dT)	variable
dNTP Mix (10mM)	1.0
MBGW	Make up to 14
Heat mixture to 65°C for 5 min on heating block and quick chill on ice for 2 min.	

Quick spin the tube and add the remaining following components to the tube

Components	Volume (µL)
5x RAS RT Buffer	4.0
RAS RT Enz	1.0
0.1 M DTT	1.0
Incubate 30-60 min at 42°C.	
Inactivate the reaction by heating at 70°C for 15 min.	

The cDNA can now be used as a template for amplification in PCR.

Example for PCR reaction

An example describing the general PCR reaction set is given below. Thus, customers are recommended to follow the instructions provided by *Taq* polymerase manufacturer.

Component	Volume (µL)
10X *PCR* Buffer	2.5
50 mM $MgCl_2$	
10 mM dNTPs	0.5
Forward primer (10 µM)	0.5
Reverse primer (10 µM)	0.5
Taq DNA Polymerase (5U/µl)	0.5
cDNA (from cDNA synthesis reaction)	0.5
Molecular Grade Water	Make up to 25

Manufactured by:

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Immediately transfer the reaction tubes from ice to pre-heated thermocycler and start thermocycling.

Recommended PCR conditions

Operation	Temp	Time	Cycles
Initial denaturation	95˚C	1-5 min	1
Denaturation	95˚C	30 s	30-40 cycles
Annealing	Tm-˚C	30-60 s	
Extension	72˚C	1 min/kb	
Final Extension	72˚C	5-10 min	--

Quality Control
cDNA synthesis
The RAS RT enzyme generated a 3 kb amplicon in presence of RAS RT buffer and gene specific primer at 42˚C.

DNase activity

No detectable change in banding pattern was observed in agarose gel after incubating the 25 units of *Taq* DNA Polymerase with 500 ng of phage DNA in RAS *Taq* reaction buffer at 37°C for 4 hrs.

RNase activity

Incubation of Human RNA (250 ng) with 25 units of *Taq* DNA polymerase in RAS *Taq* reaction buffer at 37°C for 1 hr results in no detectable change in banding pattern as determined by agarose gel electrophoresis.

E. *coli* DNA contamination

Taq polymerase is tested free of *E.coli* DNA contamination

References

1. Roth, M.J., Tanese, N. and Goff, S.P. (1985) Purification and characterization of murine retroviral reverse transcriptase expressed in Escherichia coli. J. Biol. Chem. 260, 9326–35.
2. Sambrook, J., Fritsch, E.F. and Maniatis, T. (1989) In: Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, 8.64

Presentation

Cat. No	Units	Enzyme	Buffer
ENRTE-5k	5000 units	1 x 50 µL	1 x 500µL
ENRTE-10k	10000 units	1 x 100 µL	1 x 1.0 mL
ENRTE-50k	50000 units	1 x 500 µL	1 x 5.0 mL

For further information on protocols and details for RAS MMLV reaction, please contact our technical support: info@raslifesciences.com

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